Cancer Causes and Control **11**: 129–135, 2000.

Serum tocopherols, selenium and lung cancer risk among tin miners in China

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Received 26 February 1999; accepted in revised form 8 September 1999

Key words: lung cancer, selenium, tocopherol, vitamin E.

Abstract

Objective: To evaluate the association of prediagnostic serum antioxidants and lung cancer risk we conducted a case–control study nested in an occupational cohort of tin miners.

Methods: Male workers free of cancer enrolled in the cohort. During up to 6 years of follow-up, 339 lung cancer cases were diagnosed and, among these cases, those who donated blood prospectively (n = 108) were eligible for this study. For each case, two controls alive and free of cancer at the time of case diagnosis were matched on age and date of blood collection.

Results: Overall, we observed no association between serum alpha-tocopherol, gamma-tocopherol or selenium levels and lung cancer risk. However, a significant gradient of decreasing lung cancer risk with increasing serum alpha-tocopherol was apparent for men less than 60 years old (odds ratio by tertile: 1.0, 0.9, 0.2; trend $p = 0.002$). Alpha-tocopherol was also protective in men who reported no alcohol drinking (OR by tertile: 1.0, 0.6, 0.3; trend $p = 0.008$).

Conclusion: Although there were no significant overall associations between prospectively collected serum alpha-tocopherol, gamma-tocopherol or selenium and incidence of lung cancer, results from this study suggest that higher alpha-tocopherol levels may be protective in men less than 60 years old and in those who do not drink alcohol.

Introduction

The causes of lung cancer are generally thought to be inhalants such as tobacco smoke, dusts containing carcinogens such as arsenic, and gases such as radon [1]. The Chinese tin miners of Yunnan are a high-risk cohort for lung cancer by virtue of their smoking habits and occupational exposures to radon and arsenic [2]. However, not all persons exposed to high concentrations of airborne carcinogens develop lung cancer. This is indeed true among the tin miners. It can be hypothesized that intake of dietary antioxidants and genetic makeup have the ability to neutralize or modulate carcinogen-derived oxidative radicals and that this might confer protection against lung cancer. This is presumed to be the reason that some individuals with carcinogenic exposures develop lung cancer while others do not.

Many observational studies have investigated the association between serum antioxidant levels and lung cancer [3]. The antioxidants assayed include selenium and vitamin E (alpha- and gamma-tocopherol) among others. The association between selenium and lung cancer has been inconsistent [3–15], as has the association between alpha-tocopherol levels and lung cancer [3, 6, 16–21].

The potential anti-cancer properties of tocopherols and selenium can be attributed to their antioxidant

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properties. Tocopherols function as scavengers of oxidative free radicals and protect lipids from peroxidation [22]. Tocopherols have also been reported to stimulate the immune system and consequently boost defenses against cancer cells. Selenium, in the form of selenocysteine, is a vital component of the antioxidant enzyme glutathione peroxidase, which detoxifies hydrogen peroxide and lipoperoxides [23].

In this study, we examined the association of prediagnostic serum alpha-tocopherol, gamma-tocopherol and selenium levels with subsequent risk of lung cancer (ICD-9: 162) in male Chinese tin miners. We also examined whether these associations were modified by age and alcohol intake.

Materials and methods

Study cohort

The subjects in this study are miners in the Yunnan Tin Corporation (YTC), China. The incidence rates of lung cancer are extraordinarily high in this population. Males >40 years old with underground mining experience have a crude annual incidence of over 1%. Miners aged 60–64 have an incidence rate in excess of 2.5% annually. Lung cancer represents about 80% of all cancers seen annually among YTC employees and mortality from this cancer is 10-fold higher in this area than in the rest of China [24]. For males over 50 years old the lung cancer incidence rate is 3–7 times higher than SEER rates for US males [25]. This population has been exposed to a number of known carcinogens, including tobacco smoke, radon and arsenic [26].

A longitudinal, prospective cohort study of high-risk miners of the YTC was established in 1992 with annual follow-up through 1999. Eligible high-risk miners were aged ≥35 years, with ≥10 years of underground mining and/or smelting experience, and free of cancer (except for non-melanoma skin cancer) at baseline in 1992. The baseline and follow-up activities were added to an annual YTC screening of the miners ongoing since 1973. These activities included: an interview concerning demographic, dietary, residential, occupational, and medical histories; a 24-hour food recall; chest X-ray, physical examination; and a sputum collection. The initial cohort established in 1992 comprised 6508 miners, and with each annual screen more miners entered the cohort as they reached the eligibility criteria, resulting in 9143 cohort members by 1997. During the annual screenings in 1993 and 1994, miners were asked to provide a fasting blood specimen. The sub-cohort who had blood specimens were representative of the larger high-risk cohort and comprised 45% of 9143 participants. Lung cancer cases were ascertained by reports to the Cancer Registry of the Labor Protection Institute of the YTC or from the annual screens. Over two-thirds of the cases identified were classified as squamous cell carcinomas of the lung.

Selection of cases and controls

The cases consisted of 108 men, aged 35–74 years, diagnosed with primary lung cancer (ICD-9: 162) during the years 1992–97 among those who gave blood at baseline examinations. Using incidence density sampling, the controls were selected from cohort participants who were alive and free of cancer at the time the matched case was diagnosed. Controls were matched to cases on age (±1 year) and date of blood draw (±2 weeks) in a 2:1 ratio. Selection of cases and controls was independent of assessment of exposure status.

Assessment of antioxidant levels

Serum was collected at the time of entry into the cohort (on average 2 years prior to case diagnosis) and was analyzed in case–control triplet sets. Interspersed throughout the sets were 48 masked quality-control (QC) samples (13% of study group) composed of pooled sera arranged also in sets of three to include one set per batch of analysis. Laboratory personnel at the NHANES Laboratory of Biochemical Analyses at the Centers for Disease Prevention and Control, Atlanta, were blind to the case–control status and identity of QC samples. The intra-set coefficients of variation (based on masked reference serum assays) were 2.7, 3.7 and 5.3 for alpha-tocopherol, gamma-tocopherol and selenium, respectively. The inter-batch coefficients of variation were 4.1, 3.5 and 11.4 for alpha-tocopherol, gamma-tocopherol and selenium, respectively.

Serum levels of alpha-tocopherol and gamma-tocopherol were measured by isocratic high-performance liquid chromatography with detection at three different wavelengths [27]. Selenium was measured in the serum by atomic absorption spectrometry. Quantification was based on the measurement of light absorbed at 196.0 nm by ground-state atoms of selenium from a selenium electrodeless discharge lamp (EDL) source [27].

Definition of exposures

The cumulative radon exposure estimate for each subject was obtained by summing across the estimated working level months (WLM) for each job held at the YTC prior to the date of entry at initial screening for the

high-risk cohort. The cumulative individual arsenic exposure for each job was estimated by using an index for arsenic exposure (Index of Arsenic Exposure Months or IAEM), which was calculated as a time-weighted average of arsenic concentration (mg/m^3) times exposure months ($mg/m^3 \times$ months). Individuals who had smoked cigarettes and/or pipes (water pipes or Chinese long-stem pipes) regularly for 6 months or longer at any time in their life were classified as ever smokers and were asked for information on a variety of smoking-related issues. Pack year equivalents (g/day × years ÷ 20) were used to measure cumulative tobacco consumption which was calculated separately for cigarettes (1 cigarette = 1 gram) water pipe, long-stem pipe and for total tobacco use [2]. The tobacco variable used for all analyses was the quantity of total tobacco (g/day) smoked daily. The age-adjusted relative risk estimates for lung cancer due to tobacco smoking and radon exposure were 3.6 (95% CI: 1.6–8.0) and 2.9 (95% CI: 1.3–6.8) comparing those in the highest quartile of exposure to the lowest quartile, respectively.

Trained interviewers administered a culture-specific 109-item dietary food frequency questionnaire (FFQ) in 1992, 1993 and 1995 annual screens. Miners reported the frequency of intake of foods including alcohol from grain liquor, wine and other spirits during the past year, with specification of month/season of intake when appropriate. Dietary and alcohol intake information was also obtained annually by a 24-hour food and beverage recall questionnaire. The 1992 24-hour food and beverage recall questionnaire was the primary source of dietary data presented in the current study. In order to assure the quality of the questionnaire data, a sample of the participants (2%) were re-interviewed each day by the supervisory staff. In a subsample of participants, a diet validation study comparing the diet history FFQ to 28 days of diet records was conducted in 1992–93 and in 1995–96. Deattenuated Pearson correlation coefficients of the frequency of food intake between the FFQ and food recalls were in the range −4.0 to 0.72 for both validation studies [28].

Statistical analyses

The Wilcoxon rank sum test was used to test the hypothesis that the medians of serum antioxidant concentrations were the same for cases and controls. Conditional logistic regression techniques were used to examine the association between serum antioxidants and lung cancer. Modification of the effect of antioxidants by age, season, tobacco, radon, arsenic and alcohol consumption on lung cancer risk was examined by statistical tests of the first-order interaction term in the logistic regression models. We examined the association between serum antioxidants and lung cancer stratified by age and alcohol consumption as an unmatched analysis (using unconditional logistic regression techniques) to avoid the loss of subjects due to splitting of matched sets that fell into different strata of age or alcohol consumption. All unconditional logistic regression models were adjusted for the original matching criteria (age and date of blood draw). Tertiles of exposure for each antioxidant were created using the distribution of serum antioxidants among the controls. In order to conduct linear trend analyses, variables were created using exposure scores based on the median values of each metabolite for the first to third tertiles among the controls.

Potential confounding of the association between the serum antioxidants and cancer risk by other related risk factors was explored using Spearman rank correlation analysis and multivariate logistic regression models, including stepwise regression models. If the potential confounder caused a significant change in the log-likelihood estimate ($p < 0.05$) and a greater than 10% change in the serum antioxidant beta-coefficient, it was kept in the model for further multivariate analysis. Exclusion of early cases (cases diagnosed within 1 year of blood draw) did not materially alter the risk estimates of any of the serum antioxidants. All analyses were performed using the statistical software package STATA (Texas).

Results

Cases and controls were matched closely on age and date of blood draw. Comparison of anthropometric, dietary and lifestyle variables that could be related to cancer risk yielded some differences between cases and controls (Table 1). As expected, tobacco use and radon exposure were significantly higher in the cases compared to the controls. In addition, the proportion of current smokers was much higher among the cases. The cases also were less educated, and performed mining and smelting work longer than the controls.

Prediagnostic serum antioxidant levels by case status are also shown in Table 1. Alpha-tocopherol, gamma-tocopherol and selenium levels did not differ by case–control status. The serum alpha-tocopherol, gamma-tocopherol and selenium levels ranged from 440–1658 μg/dl, 44–425 μg/dl and 20–111 ng/ml, respectively among the controls, while the serum alpha-tocopherol, gamma-tocopherol and selenium levels ranged from 316–1850 μg/dl, 40–420 μg/dl and 22–121 ng/ml, respectively among the cases.

Table 1. Selected baseline characteristics of lung cancer cases and controls[1]

Characteristic	Cases (n = 108)	Controls (n = 216)	p-Value[2]
Age (years)	63	63	0.88
BMI (kg/m^2)	21.6	21.8	0.83
School completed (years)	1	2	0.05
Monthly salary (yuan)	223	250	0.66
Age started work	16	17	0.09
Years mining/smelting jobs	31	29	0.04
Years of water-pipe smoking	46	45	0.41
Years of cigarette smoking	42	41	0.18
Cigarettes/day	10	10	0.22
All tobacco use (g/day)	18	13	0.02
Cumulative radon exposure (WLM)	510	365	0.04
Cumulative arsenic exposure as (IAEM)	10,702	9038	0.30
Retired	99 (91.7%)	194 (89.8%)	0.59
Total alcohol (g/day)[3]			
All men	87 [111]	81 [121]	0.18
Drinkers (48%)	174 [98]	171 [124]	0.39
Smoking status[4]			
Current	76 (70.4%)	119 (55.1%)	0.01
Former	16 (14.8%)	45 (20.8%)	
Never	16 (14.8%)	52 (24.1%)	
Serum			
Alpha-tocopherol (μg/dl)	778.0	773.0	0.64
Gamma-tocopherol (μg/dl)	102.5	101.5	0.97
Selenium (ng/ml)	46.5	45.0	0.75

[1] Based on unmatched data with continuous variables expressed as the median.
[2] p-Values as determined by Wilcoxon rank-sum tests and χ^2 for categorical variables.
[3] Total alcohol based on unmatched continuous data expressed as the mean [standard deviation].
[4] Smoking status shown as number of individuals in each category.

Table 2. Association between serum antioxidants and lung cancer[1]

Micro-nutrient	T1[2] OR [# cases]	T2 OR (95% CI) [# cases]	T3 OR (95% CI) [# cases]	p-Trend[3]
Alpha-tocopherol (μg/dl)[4]	1 [40]	1.3 (0.7–2.4) [38]	1.1 (0.6–2.3) [30]	0.29
Gamma-tocopherol (μg/dl)[5]	1 [33]	1.1 (0.6–2.1) [37]	1.4 (0.7–2.7) [38]	0.62
Selenium (ng/ml)[6]	1 [31]	1.2 (0.6–2.3) [38]	1.2 (0.6–2.4) [39]	0.52

[1] Adjusted for radon and tobacco exposure.
[2] First tertile used as reference group.
[3] p-Trend determined with median levels of each antioxidant across tertiles.
[4] Alpha-tocopherol tertile range: T1; 316–706 μg/dl, T2; 707–892 μg/dl, T3; 895–1850 μg/dl.
[5] Gamma-tocopherol tertile range: T1; 40–85 μg/dl, T2; 86–120 μg/dl, T3; 121–425 μg/dl.
[6] Selenium tertile range: T1; 20–39 ng/ml, T2; 40–54 ng/ml, T3; 55–121 ng/ml.

No overall significant patterns in risk for lung cancer were observed for any of the serum antioxidants analyzed (Table 2), including the various combinations of serum alpha-tocopherol, gamma-tocopherol and selenium (data not shown). Adjustment for baseline covariates, such as dietary cholesterol, vegetable, fruit, meat and alcohol consumption or arsenic exposure, which were correlated with any of the antioxidants and were possibly associated with lung cancer, did not materially change the risk estimates (data not shown).

Assessment of the modification of the effect of serum antioxidants on lung cancer risk by the case–control matching variables revealed a significant interaction between age and both serum alpha-tocopherol ($p = 0.05$) and gamma-tocopherol ($p = 0.04$). We also examined effect modification by other important exposure variables (alcohol, smoking, radon and arsenic exposure) and observed a significant interaction between alcohol consumption and alpha-tocopherol ($p = 0.04$).

Median serum antioxidant levels comparing cases to controls by age, stratified into tertiles are shown in

Table 3. Mean serum tocopherols by age tertile for lung cancer cases and controls[1]

	Cases	Controls	*p*-Value[2]
Alpha-tocopherol (μg/dl)			
< 60 years	741.0 [35]	897.5 [68]	0.01
60–65 years	866.5 [40]	812.5 [84]	0.39
> 65 years	742.0 [33]	704.0 [64]	0.25
Gamma-tocopherol (μg/dl)			
< 60 years	106.0 [35]	115.5 [68]	0.10
60–65 years	105.5 [40]	98.5 [84]	0.85
> 65 years	99.0 [33]	84.5 [64]	0.10
Selenium (ng/ml)			
< 60 years	47.0 [34]	48.0 [65]	0.93
60–65 years	46.0 [35]	44.0 [81]	0.80
> 65 years	47.0 [31]	43.0 [62]	0.58

[1] Based on unmatched data with continuous variables expressed as the median [n].
[2] *p*-Values as determined by Wilcoxon rank-sum tests.

Table 3. Serum alpha- and gamma-tocopherol levels were inversely correlated with age among the controls. In the group of individuals less than 60 years old, serum alpha-tocopherol levels were significantly lower among the cases compared to controls. Among the youngest men (40–60 years), alpha-tocopherol was significantly protective with odds of developing lung cancer 80% lower (p trend = 0.002) comparing individuals in the highest antioxidant tertile to the lowest tertile (Table 4). Adjustment for age at baseline, date of blood draw, tobacco use, arsenic and radon exposure did not materially alter the risk estimates. However, risk estimates shown were adjusted for age at baseline, date of blood draw, all tobacco use, and radon exposure.

Among those reporting no alcohol consumption, mean serum alpha-tocopherol levels were 10% lower in the cases compared to controls (739 *vs.* 822 μg/dl, respectively (p = 0.04)). Conversely, among those reporting alcohol consumption, serum alpha-tocopherol levels were 6% higher in the cases compared to controls (877 *versus* 826 μg/dl, respectively (p = 0.17)). There was no correlation between any of the three antioxidants and other risk factors such as smoking or radon exposure (data not shown).

The relative risk estimates comparing drinkers and non-drinkers are shown in Table 5. Among the non-drinkers, age, date of blood draw, all tobacco use and radon exposure adjusted odds of developing lung cancer were 70% lower in individuals in the highest tertile of serum alpha-tocopherol compared to individuals in the lowest tertile (p trend = 0.008).

Discussion

The tin miners in Yunnan, China, provide a unique population to explore the associations between serum antioxidants and risk of lung cancer in a population at very high risk for lung cancer. In addition to occupational exposures and smoking that are known causes of lung cancer, this population is also nutritionally deficient in many antioxidants [27].

Results from previous studies of serum alpha-tocopherol levels and lung cancer risk have been almost equally divided – some found lower levels of alpha-tocopherol among cases than controls while others found the opposite [3, 6, 8, 16, 18–21]. In the present study we observed no effect of vitamin E against lung cancer risk. However, when stratified by age, there was a statistically significant association among individuals in the youngest age tertile (40–60 years), where vitamin E was apparently protective.

Table 4. Association between serum tocopherols and lung cancer by age[1]

Age tertiles	T1[2] OR (95% CI) [# cases]	T2 OR (95% CI) [# cases]	T3 OR (95% CI) [# cases]	*p*-Trend[3]	*p*-Interaction
Alpha-tocopherol (μg/dl)					
< 60 years	1 [13]	0.9 (0.2–1.9) [16]	0.2 (0.06–0.76) [6]	0.002	0.05
60–65 years	1 [14]	0.6 (0.2–1.8) [8]	1.7 (0.7–4.2) [18]	0.53	
> 65 years	1 [13]	1.3 (0.5–3.9) [14]	1.7 (0.4–6.5) [6]	0.40	
Gamma-tocopherol (μg/dl)					
< 60 years	1 [10]	0.9 (0.3–2.7) [14]	0.6 (0.2–1.8) [11]	0.12	0.04
60–65 years	1 [13]	0.8 (0.3–2.1) [10]	1.5 (0.6–3.9) [17]	0.53	
> 65 years	1 [10]	2.0 (0.6–6.7) [13]	3.4 (1.0–11.9) [10]	0.09	
Selenium (ng/ml)					
< 60 years	1 [10]	0.8 (0.3–2.7) [14]	1.0 (0.3–3.2) [11]	0.93	0.75
60–65 years	1 [10]	1.5 (0.6–4.1) [10]	1.6 (0.6–4.4) [17]	0.48	
> 65 years	1 [11]	1.2 (0.4–3.4) [13]	0.8 (0.2–2.6) [10]	0.66	

[1] Adjusted for age, date of blood draw, radon and tobacco exposure.
[2] First quartile used as reference group.
[3] *p*-Trend determined with median levels of each antioxidant across tertiles.

Table 5. Association between serum alpha-tocopherol and lung cancer stratified by alcohol drinker status[1]

Alcohol	T1[2] OR [# cases]	T2 OR (95% CI) [# cases]	T3 OR (95% CI) [# cases]	*p*-Trend[3]
Alpha-tocopherol (μg/dl)				
Non-drinkers	1 [27]	0.6 (0.3–1.3) [18]	0.3 (0.1–0.7) [9]	0.008
Drinkers	1 [13]	1.7 (0.7–3.8) [20]	1.9 (0.8–4.4) [21]	0.17
Gamma-tocopherol (μg/dl)				
Non-drinkers	1 [22]	1.0 (0.4–2.4) [15]	1.1 (0.4–2.6) [17]	0.88
Drinkers	1 [11]	1.9 (0.8–4.8) [22]	2.4 (0.9–6.2) [21]	0.07
Selenium (ng/ml)				
Non-drinkers	1 [11]	0.9 (0.3–2.7) [16]	1.9 (0.7–5.0) [27]	0.12
Drinkers	1 [20]	1.2 (0.5–2.6) [22]	0.6 (0.3–1.5) [21]	0.35

[1] Adjusted for age, date of blood draw, radon and tobacco exposure.
[2] First tertile used as reference group.
[3] *p*-Trend determined with median levels of each antioxidant across tertiles.

There are very few studies investigating the association between gamma-tocopherol and lung cancer. The mean serum gamma-tocopherol levels were slightly lower among cases than controls. Among individuals in the youngest tertile, serum gamma-tocopherol was 10% lower in cases than controls; however, this difference was not statistically significant. We observed a nonsignificant inverse trend in the association between serum gamma-tocopherol and lung cancer among the individuals less than 60 years old, but comparative data from studies do not exist or have not been reported. This inverse association was essentially unchanged after adjustment for alpha-tocopherol or arsenic exposure, in addition to tobacco use and radon exposure.

The association between prospectively collected serum selenium and lung cancer has been reported in over a dozen studies [3–15]. Most of these studies found that selenium concentrations were slightly lower in the cases than in the controls except in three reports. In the current study, our cases had approximately 1% higher serum selenium than controls and we observed a positive, nonsignificant association between serum selenium and lung cancer risk.

The inverse association between serum alpha-tocopherol and gamma-tocopherol in the younger YTC men seems to originate from the inverse correlation between age and alpha-tocopherol and gamma-tocopherol levels among the controls. This is probably due to age-related impairment of absorption and transport of tocopherols. Absorption of fat-soluble vitamins is dependent on pancreatic function, biliary secretions and transport across intestinal membranes, and the efficiency of all these processes has been shown to decrease with age [29]. It has also been reported that decreases in lipoprotein lipase activity with age reduce the uptake of tocopherols by the liver and tissues from chylomicron remnants and very low-density lipoproteins that transport tocopherols [30–32]. Conversely, because younger men have lower levels of exposure to the putative carcinogens, the effects of antioxidants may be evident only at these lower carcinogen exposures.

The finding of an inverse association between serum alpha-tocopherol and lung cancer incidence among those reporting no alcohol consumption is intriguing. It has also been hypothesized that alcohol consumption may disrupt lipoproteins and reduce liver function, resulting in tocopherol "trapping", spuriously high blood tocopherol levels but tissues deprived of alpha-tocopherol. In addition, alcohol consumption may also increase the absorption of alpha-tocopherol by improving solubility and aid in its transport across the intestinal lining. Our finding of increased alpha-tocopherol in the sera of lung cancer cases among alcohol drinkers compared to cases among the non-drinkers is somewhat suggestive of tocopherol "trapping" among alcohol drinkers. Interestingly, we observed higher alpha-tocopherol levels in cases among the alcohol drinkers and not among the controls.

One of the strengths of this study is its prospective design. The collection of blood specimens and data on potential confounding factors before case diagnosis minimizes the potential for recall bias and disease effect on serum antioxidant measurements. There are also some limitations to this study. The biologic relevance of examining the relation between a few antioxidants and subsequent lung cancer incidence is a limitation of this and most similar observational studies. The serum antioxidant levels measured are also likely not indicative of lifetime serum antioxidant levels. However, they are indicators of the overall antioxidant intake and blood levels in this study population. In addition, the generalizability of these results may be somewhat restricted, because the study included only individuals exposed to mining-associated occupational pollutants. Another limitation of this study is its rather small sample size.

In summary, we found no significant overall associations between prospectively collected serum levels of alpha-tocopherol, gamma-tocopherol or selenium and lung cancer. However, we did show a dose–response reduction in lung cancer risk with increased serum alpha-tocopherol in the individuals aged between 40 and 60 years. Our findings also suggest that higher serum alpha-tocopherol is protective against lung cancer among non-drinkers of alcohol.

Acknowledgements

The authors gratefully acknowledge the support of the staff of the NHANES Laboratory of Biochemical Analyses at the Centers for Disease Prevention and Control, Atlanta, GA, USA.

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